

C L A I M S

1. A composition for the culture of pluripotent stem cells, which comprises at least one inhibitor of adenylate cyclase activity.

5 2. The composition according to claim 1, wherein the composition is a medium supplement.

3. The composition according to claim 1 or 2, wherein the composition is to proliferate pluripotent stem cells while maintaining the cells in an undifferentiated state.

10 4. The composition according to any one of claims 1 to 3, wherein the inhibitor of adenylate cyclase activity is selected from the group consisting of SQ22536 (9-(tetrahydro-2-furanyl)adenine), 2',5'-dideoxyadenosine, 9-cyclopentyladenine, 2',5'-dideoxyadenosine 3'-diphosphate, 2',5'-dideoxyadenosine 3'-monophosphate, and MDL-15 12,330A (cis-N-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine).

20 5. The composition according to any one of claims 1 to 3, wherein the inhibitor of adenylate cyclase activity is selected from the group consisting of adrenocorticotropic hormone (ACTH), brain natriuretic peptide (BNP), pituitary adenylate cyclase activating polypeptide (PACAP), and a peptide having a physiological activity substantially similar to them.

6. A medium for the culture of pluripotent stem cells, which comprises the composition according to any one of claims 1 to 5.

25 7. The medium according to claim 6, wherein the medium is free of a feeder cell, and/or serum.

8. The medium according to claim 6, wherein the medium is

free of both feeder cell and serum.

9. The medium according to any one of claims 6 to 8, wherein the medium is a minimum medium for cell culture.

10. The medium according to any one of claims 6 to 9, wherein the medium comprises further a differentiation inhibitory factor, a serum replacement and an antioxidant.

11. A process for the culture of pluripotent stem cells, which comprises culturing the pluripotent stem cells under a condition such that adenylate cyclase activity is inhibited, said process allowing the pluripotent stem cells to proliferate or establish while maintaining the cells in an undifferentiated state.

12. The process according to claim 11, wherein the condition such that adenylate cyclase activity is inhibited involves the use of an inhibitor of adenylate cyclase activity.

13. The process according to claim 11 or 12, wherein the culture process is performed using the medium according to any one of claims 6 to 10.

14. The process according to claim 11 or 13, wherein the pluripotent stem cells are ES cells.

15. The process according to claim 11 or 13, wherein the pluripotent stem cells are derived from a mammal.

16. The process according to claim 11 or 13, wherein the pluripotent stem cells are derived from a human.

17. A process for the preparation of a clonal population of undifferentiated pluripotent stem cells, which comprises culturing the undifferentiated pluripotent stem cells under a condition such that

adenylate cyclase activity is inhibited.

18. A process for the preparation of a clonal population of undifferentiated pluripotent stem cells, which comprises isolating undifferentiated pluripotent stem cells from a living body, and culturing the undifferentiated pluripotent stem cells under a condition such that adenylate cyclase activity is inhibited.

19. The process according to claim 17 or 18, wherein the condition such that adenylate cyclase activity is inhibited involves the use of an inhibitor of adenylate cyclase activity.

10. 20. The process according to any one of claims 17 to 19, wherein the culture process is performed using the medium according to any one of claims 6 to 10.

15. 21. The process according to any one of claims 17 to 20, wherein one pluripotent stem cell is cultured to provide a clonal population of the cells.

20. 22. The process according to any one of claims 17 to 21, wherein pluripotent stem cells are cultured in the medium according to claim 7 or 8 to provide a clonal population of the cells, in which the pluripotent stem cells are seeded at a lower density than that which allows adjacent pluripotent stem cells to interact with each other, so as to induce the proliferation of undifferentiated pluripotent stem cells.

23. The process according to any one of claims 17 to 22, wherein one pluripotent stem cell is cultured in the medium according to claim 7 or 8 to provide a clonal population of the cells.

25. 24. The process according to any one of claims 17 to 23, wherein the pluripotent stem cells are ES cells.

25. The process according to any one of claims 17 to 24, wherein the pluripotent stem cells are derived from a mammal.

26. The process according to any one of claims 17 to 25, wherein the pluripotent stem cells are derived from a human.

5 27. A clonal population of undifferentiated pluripotent stem cells, which is obtainable by the process according to any one of claims 17 to 26.

10 28. Use of an inhibitor of adenylate cyclase activity, for culturing pluripotent stem cells while maintaining the cells in an undifferentiated state to proliferate or establish the undifferentiated cells.

15 29. Use of a composition comprising an inhibitor of adenylate cyclase activity, for culturing pluripotent stem cells while maintaining the cells in an undifferentiated state to proliferate or establish the undifferentiated cells.